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(54) Title: METHODS OF USING FARNESOID X RECEPTOR (FXR) AGONISTS

(57) Abstract: Treatment of human hepatocytes with farnesoid X receptor (FXR) agonists resultedin increased expression of FGF-19. Methods of using FXR agonists to alter cell metabolism, and in pharmaceutical weight loss methods, are described.

METHODS OF USING FARNESOID X RECEPTOR (FXR) AGONISTS

Field of the Invention

The present invention relates to farnesoid X receptor (FXR) agonists and their use in methods of affecting the metabolism of cells, and in pharmaceutical weight loss methods.

Background of the Invention

Being overweight or obese substantially raises an individual's risk of morbidity from hypertension, dyslipidemia, type 2 diabetes, coronary heart disease, and other conditions. Despite the expected medical benefits, many overweight individuals find it difficult to successfully lose weight by diet management alone. Obesity is recognized as a complex multifactorial condition that develops from the interaction of genetic and environmental factors. See, e.g., Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults, Am. J. Clin. Nutr. 68:899 (1998).

Various pharmaceutical compounds have been utilized in weight loss treatments. Serotonergic agents that inhibit the reuptake of serotonin are reported to act on the hypothalamus to decrease satiety. However, serious cardiovascular side effects have been reported in some individuals treated with such agents. Fenfluramine and dexfenfluramine, serotonergic agents previously utilized in the United States for the treatment of obesity, have been withdrawn from the U.S. market due to reports of valvular heart disease and primary pulmonary hypertension. (Davidoff et al., Arch Intern Med 161:1429 (2001); Michelakis et al., Am J Med Sci 321:292 (2001); Weissman, Am J Med Sci 321:285 (2001); 2001 PHYSICIANS DESK REFERENCE®, Medical Economics Co., (2000)).

In view of the need for medical weight loss therapies, additional pharmaceutical methods useful in weight control or weight loss are desirable.

Summary of the Invention

A first aspect of the present invention is a method of increasing leptin release from the adipocyte cells of a mammalian subject, by administering an FXR agonist to the subject. Leptin release is increased, compared to the leptin release that would occur without FXR agonist administration.

A further aspect of the present invention is a method of decreasing glucose uptake by the adipocyte cells of a mammalian subject, by administering an FXR agonist to the subject. Glucose uptake is decreased compared to that which would occur in the absence of FXR agonist administration.

A further aspect of the present invention is a method of treating a mammalian subject to achieve weight loss, by administration of a pharmaceutically acceptable FXR agonist. The subject's weight is decreased, compared to that which would occur in the absence of FXR agonist treatment.

A further aspect of the present invention is a method of reducing the total body mass of a mammalian subject, by administering a pharmaceutically acceptable FXR agonist. The subject's total body mass is reduced compared to the subject's total body mass that would occur in the absence of FXR agonist treatment.

A further aspect of the present invention is a method of increasing the metabolic rate of a mammalian subject, by administering a pharmaceutically acceptable FXR agonist. The metabolic rate of the subject is increased compared to the rate that would occur in the absence of FXR agonist treatment.

A further aspect of the present invention is a method of increasing serum leptin in a mammalian subject, by administering to a subject a pharmaceutically acceptable FXR agonist. The serum leptin in the subject is thereby increased compared to that would occur in the absence of FXR agonist treatment.

A further aspect of the present invention is a method of inducing expression of FGF19 in a human hepatocyte cell, by administering an FXR agonist to the cell. The cell may be *in vitro*.

Detailed Description of the Invention

The present invention relates to the use of Farnesoid X Receptor (FXR) agonists to affect the metabolism of cells, and as a pharmaceutical treatment for weight control and weight loss. The present inventors determined that activation of the nuclear receptor FXR by a bile acid agonist, as well as by a small molecule FXR agonist, caused an increase in transcription of a human Fibroblast Growth Factor gene (hFGF19), leading to an increase in the quantity of mRNA encoding the fibroblast growth factor. Accordingly, in humans expression of FGF19 and its downstream activity can be modulated using FXR agonists. Human FGF19 has been reported to induce leptin release from rat adipocyte cells and decrease glucose uptake by rat adipocyte cells; transgenic mice expressing hFGF19 have been reported to be less fat than their nontransgenic littermates; increased oxygen consumption has been reported in mice administered recombinant FGF-19; and administration of FGF-19 has been suggested as a treatment for obesity (WO 01/18210, Genentech).

FXR

FXR is a member of the nuclear receptor family of ligand-activated transcription factors that includes receptors for the steroid, retinoid, and thyroid hormones (DJ. Mangelsdorf, et al., *Cell* 83:841-850 (1995)). Northern and *in situ* analysis show that FXR is most abundantly expressed in the liver, intestine, kidney, and adrenal (BM. Forman, et al., *Cell* 81:687 (1995) and W. Seol, et al., *Mol. Endocrinnol.* 9:72 (1995)). FXR binds to DNA as a heterodimer with the 9-cis retinoic acid receptor (RXR). The FXR/RXR heterodimer preferentially binds to response elements composed of two nuclear receptor half sites of the consensus AG(G/T)TCA organized as an inverted repeat and separated by a single nucleotide (IR-1 motif) (BM. Forman, et al., *Cell* 81:687 (1995)). An early report showed that rat FXR is activated by micromolar concentrations of farnesoids such as farnesol and juvenile hormone (BM. Forman, et al., *Cell* **81**:687-693 (1995)). However, these compounds failed to activate the mouse and human FXR, leaving the nature of the endogenous FXR ligand in doubt. Several naturally-occurring bile acids bind to and activate FXR at physiological concentrations (PCT WO 00/37077, published 29 June 2000)). As discussed therein, the bile

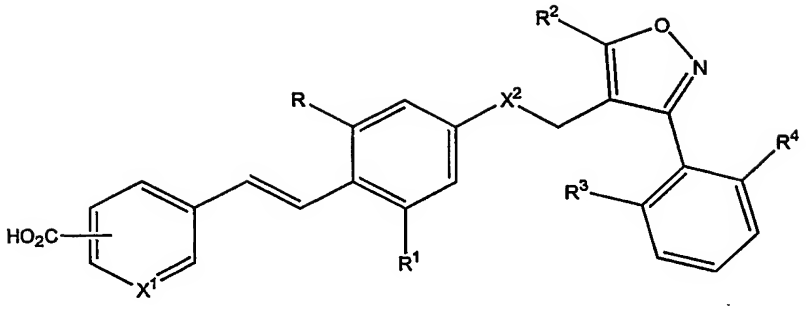
acids that serve as FXR ligands include chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), lithocholic acid (LCA), and the taurine and glycine conjugates of these bile acids.

Bile acids are cholesterol metabolites that are formed in the liver and secreted into the duodenum of the intestine, where they have roles in the solubilization and absorption of dietary lipids and vitamins. Most bile acids (~95%) are subsequently reabsorbed in the ileum and returned to the liver via the enterohepatic circulatory system. The conversion of cholesterol to bile acids in the liver is under feedback regulation: bile acids down-regulate the transcription of cytochrome P450 7a (CYP7a), which encodes the enzyme that catalyzes the rate limiting step in bile acid biosynthesis. FXR is involved in both the stimulation and the repression (via CYP7a) of target genes involved in bile acid and cholesterol homeostasis.

FXR ligands

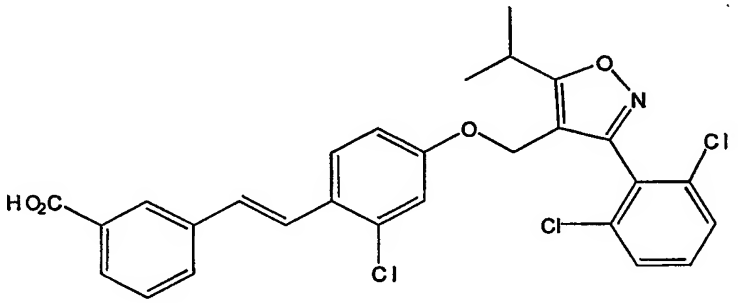
The bile acids chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), lithocholic acid (LCA), and the taurine and glycine conjugates thereof selectively activate FXR (WO 0037077, Glaxo Group Limited). As used herein, the term "FXR agonist" refers to compounds that achieve at least about 50% activation of FXR relative to CDCA, the appropriate positive control in the assay methods described in PCT Publication No. WO 00/37077 published 29 June 2000 to Glaxo Group Limited, the subject matter of which is incorporated herein by reference in its entirety. Preferably, the FXR agonist compounds used in the methods of this invention achieve at least about 70% activation of FXR in the scintillation proximity assay or the HTRF assay as described in PCT Publication No. WO 00/37077; more preferably, the compounds achieve at least about 80%, 90%, 95%, 97% or greater activation of FXR in the scintillation proximity assay or the HTRF assay as described in PCT Publication No. WO 00/37077.

An FXR agonist for use in the present invention is the compound known as GW4064, as disclosed in PCT Publication No. WO 00/37077 published 29 June 2000 to Glaxo Group Limited, which describes FXR ligand compounds characterized by the following formula (I)

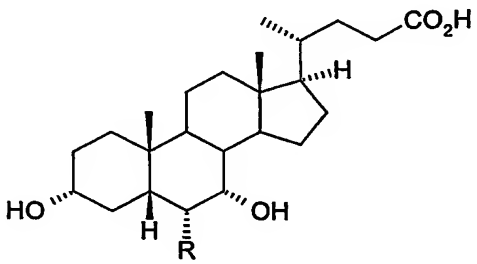


wherein X^1 is CH or N; X^2 is O or NH; R and R^1 may independently be H, lower alkyl, halogen, or CF_3; $R^2$is lower alkyl; R^3 and R^4 may independently be H, lower alkyl, halogen, CF_3, OH, O-alkyl, or O-polyhaloalkyl.

GW4064, an example of a compound of Formula (I), is a potent and selective FXR ligand and has the following formula (II):



FXR agonists for use in the present invention further include compounds of formula III:



(III)

wherein R is ethyl, propyl or allyl, and pharmaceutically acceptable salts, solvates or amino acid conjugates thereof.

Suitable pharmaceutically acceptable salts of the above compounds may be readily determined by one skilled in the art and may include, for example, basic salts such as metallic salts made from aluminium, calcium, lithium, magnesium, potassium, sodium, and zinc or organic salts made from N,N'-dibenzylethylenediamine, chlorprocaine, choline, diethanolamine, ethylendiamine, meglumine (N-methylglucamine), and procaine. Such salts may be prepared using conventional techniques, as are known in the art. As used herein, the term "solvate" is a crystal form containing the active compound (or a pharmaceutically acceptable salt thereof) and either a stoichiometric or a non-stoichiometric amount of a solvent. Solvents, by way of example, include water, methanol, ethanol, or acetic acid.

As used herein, the term "amino acid conjugates" refers to conjugates of a compound with any suitable amino acid. Preferably, such suitable amino acid conjugates have the added advantage of enhanced integrity in bile or intestinal fluids. Suitable amino acids include but are not limited to glycine and taurine. Thus, the present invention encompasses the use of glycine and taurine conjugates of FXR agonists.

Compounds of formula (III) include compounds selected from the group consisting of 3α,7α-dihydroxy-6α-ethyl-5β-cholan-24-oic acid; 3α,7α-dihydroxy-6α-propyl-5β-cholan-24-oic acid and 3α,7α-dihydroxy-6α-allyl-5β-cholan-24-oic acid and their pharmaceutically acceptable salts, solvates or amino acid conjugates thereof.

The amount of FXR agonist, or pharmaceutically acceptable salt or solvate thereof, which is required to achieve the desired biological effect will depend on a number of factors such as the means of administration, the desired outcome, and the recipient. In general, in treating mammals for weight control or weight loss purposes, a typical daily dose of an FXR agonist of formula (I-III) may be expected to lie in the range of from about 0.01 mg/kg to about 100 mg/kg. This dose may be administered as a single unit dose or as several separate unit doses or as a continuous infusion.

FGF19

FGF19 is a member of the fibroblast growth factor (FGF) family of proteins. Members of this family are known to be involved in tissue repair, angiogenesis, induction of

genes containing an FGF-inducible response element (FiRE), mitogenesis, oncogenesis, and differentiation. The biological specificity of FGFs is believed to be partly due to the controlled expression of both the FGFs and the FGF receptors (FGFRs). Four related receptor tyrosine kinases have been identified that bind to members of the FGF family; the presence of heparin or heparan sulfate proteoglycans is believed to be required for the biological activity of FGFs.

The structure and expression of human FGF-19 is described in Nishimura et al., Biochim Biophys Acta 1444:148 (1999). FGF-19 binds with high affinity to the cell surface tyrosine kinase receptor FGF Receptor 4 (FGFR4), and displays selective binding to FGFR4. Xie et al., Cytokine 11:729 (1999)..

The sequence for human FGF19 mRNA is provided at GenBank Accession Number AF110400:

CDS 464-1114

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gctcccagcc aagaacctcg gggccgctgc gcggtgggga ggagttcccc gaaacccggc  60
cgctaagcga ggcctcctcc tcccgcagat ccgaacggcc tgggcggggt caccccggct 120
gggacaagaa gccgccgcct gcctgcccgg gcccggggag ggggctgggg ctggggccgg 180
aggcggggtg tgagtgggtg tgtgcggggg gcggaggctt gatgcaatcc cgataagaaa 240
tgctcgggtg tcttgggcac ctacccgtgg ggcccgtaag gcgctactat ataaggctgc 300
cggcccggag ccgccgcgcc gtcagagcag gagcgctgcg tccaggatct agggccacga 360
ccatcccaac ccggcactca cagccccgca gcgcatcccg gtcgccgccc agcctcccgc 420
acccccatcg ccggagctgc gccgagagcc ccagggaggt gccatgcgga gcgggtgtgt 480
ggtggtccac gtatggatcc tggccggcct ctggctggcc gtggccgggc gccccctcgc 540
cttctcggac gcggggcccc acgtgcacta cggctggggc gaccccatcc gcctgcggca 600
cctgtacacc tccggccccc acgggctctc cagctgcttc ctgcgcatcc gtgccgacgg 660
cgtcgtggac tgcgcgcggg gccagagcgc gcacagtttg ctggagatca aggcagtcgc 720
tctgcggacc gtggccatca agggcgtgca cagcgtgcgg tacctctgca tgggcgccga 780
cggcaagatg caggggctgc ttcagtactc ggaggaagac tgtgctttcg aggaggagat 840
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tgctgccagg ggctgtggta cctgcagcgt gggggacgtg cttctacaag aacagtcctg 1200
agtccacgtt ctgtttagct ttaggaagaa acatctagaa gttgtacata ttcagagttt 1260
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cagaagacag gcagtagttt taatttcagg aacaggtgat ccactctgta aaacagcagg 1560
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(SEQ ID NO:1)

Human FGF19 polypeptides and nucleic acid molecules encoding the same are described in WO 0118210 (Genentech, Inc.), EP1032668 (Genentech), and WO 0118209 (Curagen Corporation). The production of transgenic mice expressing human FGF19 (using the promoter for myosin light chain to result in muscle specific transcription of the transgene) is reported in WO 0118210, where it is further reported that these mice demonstrated increased food intake and increased metabolic rate (evidenced by their rate of oxygen consumption and increased urine output). However, the transgenic mice weighed significantly less than their non-transgenic littermates, despite their increased food intake. The transgenic mice had normal linear growth, and normal body temperature and bone length. It is postulated in WO0118210 that the decreased body weight of transgenic hFGF19 mice is due to decreased adiposity; leptin (which is reported to correlate closely with adipose tissue mass in humans and rodents) is decreased in the transgenic mice. Infusion of FGF19 to non-transgenic mice was reported to cause an increase in food intake. It is stated in WO0118210 that FGF19 decreases adiposity without altering muscle mass or long bone formation, and that FGF19 is indicated as a therapeutic in the treatment of obesity and related conditions. Further, the effects of a high-fat diet on glucose tolerance in transgenic mice expressing hFGF19 were compared to the effects in non-transgenic littermates. Transgenic mice fed a high-fat diet for ten weeks were subjected to a glucose tolerance test; the majority of the non-transgenic mice fed a high-fat diet were defined as diabetic, whereas none of the transgenic mice fed a comparable high-fat diet were defined as diabetic by the glucose tolerance test. WO 01/18210.

Definitions

"Mammal" as used herein includes primates and humans, as well as livestock and companion animals.

"Pharmaceutical weight loss treatment" as used herein refers to administration of a pharmaceutical compound to a subject whose weight is greater than a medically acceptable or

medically desirable amount, to achieve a reduction in the subject's weight. "Pharmaceutical treatment of obesity" is an aspect of pharmaceutical weight loss treatment and refers to such treatment for individuals whose body mass meets an accepted medical definition of obesity. One commonly accepted measure of overweight in humans is the Body Mass Index (BMI); overweight may be defined as a BMI of at least 25 kg/m^2, with obesity defined as a BMI of at least $30kg/m^2$. Pharmaceutical weight loss treatment may be accompanied by a change in diet and/or other behavioral modifications such as support groups and/or patient education. As used herein, pharmaceutical weight loss treatment does not imply a "cure" for obesity or permanent weight loss. "Pharmaceutical weight maintenance treatment" as used herein refers to administration of a pharmaceutical compound to a subject as an aid in maintaining a desired weight. As described herein, FXR agonists are useful in such treatments described above.

Body Mass Index is a numerical measurement of relative weight for height, and has been significantly correlated with total body fat content. BMI is calculated as weight (kg)/height squared (m^2). See, e.g., Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults. Am. J. Clin. Nutr. 68:899 (1998).

As used herein, an FXR ligand pharmaceutical compound for the treatment of obesity or for weight loss treatment is one in which administration (in an appropriate pharmaceutical formulation and in a therapeutically effective amount) to a mammal, and preferably to humans, has been shown to increase weight loss over time, compared to the change in weight that would have occurred had the subject not been administered the compound. Therapeutically effective amounts of such compounds for use in treatment of obesity or for weight loss can be readily determined by those skilled in the art using, e.g., dose-response studies.

Treatment of a subject with an FXR agonist pharmaceutical compound comprises administration of an effective amount (for the condition being treated) of the pharmaceutical agent to a subject. The dose of agent is determined according to methods known and accepted in the pharmaceutical arts, and can be determined by those skilled in the art.

Differential Gene Expression

The present study utilized differential gene expression analysis as performed by CuraGen Corporation (New Haven, CT) using transcript profiling technology. See *Nat Biotechnol* 17:798-803, 1999; see also US Patent No. 5,871,697 and 5,972,693. In brief, RNA is extracted from samples and cDNA synthesis is carried out. The cDNAs are then digested with multiple pairs of different restriction enzymes (standard set of 96 different pairs of restriction enzymes with 6 base-pair recognition sites (subsequence pairs)), and the digested products are ligated to complementary adapters containing standardized PCR priming sequences. Multiple PCR cycles are carried out using one biotinylated adapter-specific primer and one fluorescently labeled adapter-specific primer. Following PCR amplification, the biotin-labeled DNA is purified on immobilized streptavidin. Denatured single stranded cDNA fragments are then sized using capillary electrophoresis, and the fluorescently labeled fragments are detected by laser excitation. Since the biotin label is needed for purification and the fluorescent label is needed for detection, all analyzed fragments result from restriction digestion with both enzymes.

Differentially expressed peaks are identified by comparing the composite traces of the experimental samples vs. the control samples using bioinformatics algorithms. Each differentially expressed peak is then compared to a database containing a 'virtual' restriction digest of a human sequence database. (The database was constructed by performing *in silico* digests on a human sequence database, using all subsequence pairs as described above; for each transcribed DNA sequence, the computed fragment sizes and associated restriction enzymes (bands) are stored in the database.) By comparing the differentially expressed peaks with the database, genes are identified that are predicted to generate DNA fragments with lengths and terminal sequences that match the restriction enzymes used and the detected length of the differentially expressed peaks. A single differentially expressed band may be part of one or more genes. Additional bands from at least one of these genes should be present and should also be differentially expressed.

Thus, confirmation and gene identification of differentially expressed bands can occur by two routes. One method is GeneCalling/Poisoning. In this case, cDNA fragments

representing differentially expressed genes can be identified by database searching with the 6 base-pair restriction enzyme recognition sequences at the fragment ends and the exact length of each fragment (determined electrophoretically, subtracting linker length). Database searching for genes predicted to have restriction fragments of matching lengths enables the identification of such genes whose sequences reside in that database (a "GeneCall"). The detection of multiple fragments derived from the same gene which show differential expression of the same directional modulation increases the likelihood that the prediction of the gene identity is correct. The differentially expressed gene fragment and the predicted gene sequence identified by the database lookup, can then be linked through a positive poisoning reaction. In this process, the reaction containing the fragment of interest is performed a second time using the same end primers, but in the presence or absence of an excess of an unlabeled oligonucleotide whose sequence is derived from the predicted gene fragment. If the identity of the fragment was predicted correctly, the unlabeled oligonucleotide will out-compete the universal oligonucleotide for priming that fragment and, in the resulting chromatogram, will appear to ablate that peak specifically without affecting the amplification of the other fragments.

An alternative method for gene identification and confirmation, Isolation/Poisoning, relies on the isolation of the differentially expressed fragment from the re-amplified GeneCalling chemistry reaction from a preparative gel. The gel-purified fragment is re-amplified and cloned in a standard PCR product cloning vector. The insert is sized and sequenced, and primers for poisoning are designed from one or both ends of the cloned fragment. The poisoning reaction is performed and analyzed as described above. Successful ablation of the peak using the unlabeled oligonucleotide based on the cloned sequence identifies the sequence as corresponding to the original differentially expressed gene fragment. Subsequently, the gene identification is obtained by standard BLASTN or BLASTX analysis of the poisoning cloned sequence.

The present invention provides methods of using FXR agonists to modulate FGF-19 expression by mammalian cells and, in mammalian organisms, to thereby modulate the downstream effects of FGF-19 . The present methods further provide methods of using FXR

agonists to modulate the metabolism of mammals , and as a treatment for weight loss or weight control in mammals. Such methods include using FXR agonist administration to a mammal to achieve an increase in circulating or serum leptin levels. The increase in leptin levels is compared to that which would otherwise occur (i.e., occur in the absence of the FXR agonist administration).

While not wishing to be held to a single theory, the present inventors believe that administration of an FXR agonist results in increased expression of genes that play a role in metabolism and weight maintenance. FXR agonists are shown herein to increase expression of FGF19 in human cells (hepatocytes). A protein homologous to FGF19, or having high sequence similarity to FGF19, may not naturally occur in all mammals. However it is postulated that non-human mammals possess fibroblast growth factors (or biomolecules having similar functional effects) that provide an FXR-mediated pathway similar to the FXR-FGF19 pathway, such that administration of FXR agonists to the animal will affect adipocyte function, metabolism and/or overall weight maintenance (or weight loss) in the manner described herein.

Leptin is a hydrophilic protein secreted from white adipocytes. Administration of recombinant leptin to *ob/ob* mice, which lack the functional protein and are obese, results in a reduction of food intake and an increase in energy expenditure. Halas et al., Science 269: 540-549 (1995). Insulin, glucocorticoids, TNFα, and interleukin-1 have been reported to stimulate the expression of the *ob* gene; fasting and the administration of isoprenaline or selective β3-adrenoceptor agonists have been reported to cause a decrease in *ob* gene expression and a corresponding decrease in circulating leptin. Serum concentrations of leptin have been reported as reflecting the nutritional status and body fat mass of individuals.

The present invention further provides a method of decreasing glucose uptake by the cells of a mammal (particularly adipocytes or white adipocyte cells) by administering an FXR agonist to the mammal in an amount effective to decrease glucose uptake by such cells. The decrease in glucose uptake is relative to that which would occur in the absence of the FXR agonist treatment.

The present invention further provides a method of treating a mammalian subject in need of weight loss or weight maintenance treatment, by administering to the subject a pharmaceutically acceptable FXR agonist in an amount effective to decrease said subject's

weight. The decrease in weight is relative to the change in weight which would occur (or would be expected to occur) in the absence of FXR agonist treatment. The present methods include pharmaceutical weight loss treatment of humans.

The present uses of FXR agonists include methods of increasing the metabolic rate of a mammalian subject, or increasing serum (or circulating) leptin in a mammalian subject, comprising administering to a subject a pharmaceutically acceptable FXR agonist in an amount effective to increase the metabolic rate or increase the serum leptin concentrations of the subject. The increase in metabolic rate may be assessed by any suitable means as is known in the art, such as by measuring oxygen consumption and/or urine output in a controlled setting. The increase in metabolic rate or leptin concentration is relative to that would occur in the absence of FXR agonist treatment.

While not wishing to be held to a single theory underlying the present invention, the present inventors believe that, in a mammal in which both Farnesoid X Receptors and FGF19 (or a protein having similar function to FGF19) are naturally present, administration of an FXR agonist to the animal will increase levels of the fibroblast growth factor (or similar protein), will affect adipocytes (including but not limited to increased release of leptin and/or decreased uptake of glucose), and will affect the animal's overall metabolic and/or weight status.

Also provided herein is a method of inducing FGF19 expression from human cells, preferably human hepatocytes, by administering an FXR agonist to said cell or exposing said cell to an FXR agonist; the cells may be *in vitro*. FGF19 protein secreted into cell culture media may be isolated and purified using any suitable technique as is known in the art. All publications and references, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference in their entirety as if each individual publication or reference were specifically and individually indicated to be incorporated by reference herein as being fully set forth. Any patent application to which this application claims priority is also incorporated by reference herein in its entirety in the manner described above for publications and references.

EXAMPLES

Example 1

Differential Expression of FGF19 in Human Hepatocytes Treated with FXR Agonists

The pattern of gene expression induced in human hepatocytes after treatment with an FXR ligand was measured.

Materials and Methods

Nine samples of human hepatocytes were used: Human hepatocytes treated with DMSO control (3 samples), human hepatocytes treated with chenodeoxycholic acid (CDCA; 3 samples), and human hepatocytes treated with FXR agonist GW4064X (3 samples). RNA was extracted from the cells and gene expression analysis was performed by CuraGen Corporation (New Haven, CT) using transcript profiling technology as described above. See *Nat Biotechnol* 17:798-803, 1999; see also US Patent No. 5,871,697 and 5,972,693.

Three independent reactions from each cDNA sample were compared for quality of electrophoretic peak resolution and reproducibility of peak patterns. Composite traces from each sample were generated, and then compared among the three independent samples for peak quality and reproducibility. The resulting traces represent the total gene expression profile for the tissue sampled and treatment. The databases for each sample were compared to identify differences in gene expression resulting from the different treatments. The composite traces calculated for each sample group, based on average peak height and variance, were compared among sample groups using software designed to identify peaks representing differential expression.

Bands (representing gene fragments) that did not change expression levels in either of the treatments (compared to control group) were filtered out. Bands showing differential expression in at least one of the treatments by a factor greater than or equal to +/- 1.5 fold (compared to control) were included in the analysis.

Results

Expression of human Fibroblast Growth Factor 19 (hFGF19) was increased 36.4-fold in human hepatocytes treated with FXR agonist compound GW4064X compared to control

cells; expression of hFGF19 was increased 5.6-fold in human hepatocytes treated with CDCA compared to control cells. These results indicated that treatment of a cell with an FXR agonist increases transcription of hFGF19.

Example 2

Effect of FXR Agonist on Leptin Release from Adipocytes in vitro

The addition of recombinant human FGF-19 to cultures of primary rat adipocytes is reported to increase the release of leptin from the cells (WO 0118210). As noted in Example 1, herein, expression of human Fibroblast Growth Factor 19 (hFGF19) was increased in human hepatocytes treated with FXR agonist compounds, compared to control cells.

The present study investigates the use of an FXR agonist to induce expression of FGF-19 in liver cells. Increased secretion of the hFGF19 protein can thereby increase the release of leptin from adipocytes.

Cultures of rat adipocytes are established using any suitable means as is known in the art. One suitable method harvests fibroblastic preadipocytes from the inguinal fat deposit of sucking rats, which are then cultured and induced to differentiate into mature adipocytes. Following differentiation, the *ob* (leptin) gene is expressed and leptin is secreted into the culture medium (typically by day 4 after induction). Mitchell et al., Biochem Biophys Res Comm, 230:360 (1997). Alternatively, isolated primary adipocytes may be cultured (see, e.g., Hardie et al., Horm Metab Res 28:685 (1996)), or excised pieces of adipose tissue may be maintained in cell culture conditions (see, e.g., Ott et al., Exp. Biol. Med. 226(9):841 (2002)).

An FXR agonist is added to liver cells cultures (primary human hepatocytes or HuH7 cells) in order to induce expression and secretion of FGF19. Suitable FXR agonists include compounds of Formulas I-III as described herein. The resulting conditioned media containing FGF19 is then added to adipocyte cell cultures in a range of concentrations. Control cell cultures are also prepared (without FXR agonist), to provide control media which is added to control cultures of adipocyte cells.

Leptin secreted into culture medium from the adipocyte cell cultures is measured by any suitable means as is known in the art, including e.g., Enzyme Linked Immunosorbent Assay (ELISA) or Radio-Immunoassay (RIA) (Rat Leptin RIA kit, Linco Research Inc., St. Charles, MO). Leptin secretion is measured as a function of FXR agonist concentration, (and/or conditioned media concentration, and/or FGF19 concentration) and time elapsed after addition of conditioned media to adipocyte cell cultures, and is compared to control cell cultures.

Example 3

Effect of FXR Agonist on Glucose Uptake by Adipocytes in vitro

The addition of recombinant human FGF-19 to cultures of primary rat adipocytes has been reported to decrease the uptake of glucose (WO 0118210). The present study investigates the use of an FXR agonist to induce expression of FGF-19 in liver cells, and the effect of hFGF19 protein on the uptake of glucose by rat white adipocytes.

Cultures of primary rat adipocytes and human liver cells are established using any suitable means as is known in the art, as discussed above.

An FXR agonist is added to liver cell cultures (primary human hepatocytes or HuH7 cells) to induce expression and secretion of FGF19. Control liver cell cultures (without exposure to FXR agonist) are also prepared. Suitable FXR agonists include compounds of Formulas I-III as described herein. Conditioned media obtained from the liver cell cultures exposed to FXR agonist (and control medium) are then added to adipocyte cell cultures in a range of concentrations.. Glucose uptake by adipocytes is measured by any suitable means as is known in the art, as a function of FXR agonist concentration (and/or conditioned media concentration, and/or FGF19 concentration) and time elapsed after addition of FXR agonist, and is compared to control cell cultures.

Example 4

FXR Agonist in Mice

Infusion of FvB mice with recombinant human FGF-19 (1mg/kg/day, delivered intravenously by an osmotically driven implanted pump) has been reported to result in

increased food intake and increased oxygen consumption compared to mice infused with carrier alone (WO 01 18210, Genentech).

Non-transgenic FvB mice are administered an FXR agonist in a suitable carrier; control mice are administered the carrier alone. Suitable FXR agonists include compounds of Formulas I-III as described herein. Administration may be by any means suitable for the particular FXR agonist (e.g., oral, subcutaneous, intravenous, or via implanted pump). Mice receiving FXR agonist may be further divided into groups receiving varying dosage regimes of FXR agonist. Treated and control mice may be further divided according to feeding regime (e.g., ad libitum normal diet; controlled portion normal diet; ad libitum high fat diet; controlled portion high fat diet).

Weight, and optionally oxygen consumption, of the treated mice and the controls are measured at various time points and compared. Weight may be measured by any suitable means, including total animal weight and/or sacrifice of the animals after a set amount of time and measurement of the weight of specific fat deposits (e.g., epididymal, retroperitoneal with peri-renal). Circulating leptin may also be measured at various time points and compared among treatment group(s) and controls. A decrease in weight, or decreased rate of weight gain, and/or decrease in adiposity (as indicated by circulating leptin levels), in treated mice relative to controls indicates that the FXR agonist decreases adiposity, and indicates FXR agonists as a therapeutic in the treatment of obesity in mammals.

Example 5

FXR Agonist in Rats

A strain of rat suitable for use in laboratory experiments and not known to have metabolic defects are administered an FXR agonist in a suitable carrier and at a range of FXR dosages; control rats are administered the carrier alone. Suitable FXR agonists include compounds of Formulas I-III as described herein. Administration may be by any means suitable for the particular FXR agonist (e.g., oral, subcutaneous, intravenous, or via implanted pump). Treated and control rats may be further divided according to feeding regime (e.g., ad libitum normal diet; controlled portion normal diet; ad libitum high fat diet; controlled portion high fat diet).

Weight, and optionally oxygen consumption, of the treated animals and the controls are measured at various time points and compared. Weight may be measured by any suitable means, including total animal weight and/or sacrifice of the animals after a set amount of time and measurement of the weight of specific fat deposits (e.g., epididymal, retroperitoneal with peri-renal). Circulating leptin may also be measured at various time points and compared among treatment group(s) and controls. A decrease in weight, or decreased rate of weight gain, and/or decrease in adiposity (as indicated by circulating leptin levels), in treated animals relative to controls indicates that the FXR agonist decreases adiposity, and indicates FXR agonists as a therapeutic in the treatment of obesity in mammals.

Example 6

FXR Agonist in Primates

A group of non-human primates suitable for use in laboratory experiments and not known to have any metabolic defects are administered an FXR agonist in a suitable carrier and at a range of FXR dosages; control primates are administered the carrier alone. Suitable FXR agonists include compounds of Formulas I-III as described herein. Administration may be by any means suitable for the particular FXR agonist (e.g., oral, subcutaneous, intravenous, or via implanted pump). Treated and control animals may be further divided according to feeding regime (e.g., ad libitum normal diet; controlled portion normal diet; ad libitum high fat diet; controlled portion high fat diet).

Weight, and optionally oxygen consumption, of the treated animals and the controls are measured at various time points and compared. Weight may be measured by any suitable means, including total animal weight and/or sacrifice of the animals after a set amount of time and measurement of the weight of specific fat deposits (e.g., epididymal, retroperitoneal with peri-renal). Circulating leptin or expression of FGF19 may also be measured at various time points and comparedamong treatment group(s) and controls. A decrease in weight, or decreased rate of weight gain, and/or decrease in adiposity (as indicated by circulating leptin levels), in treated animals relative to controls indicates that the FXR agonist decreases adiposity, and indicates FXR agonists as a therapeutic in the treatment of obesity in mammals.

That which is claimed is:

1. A method of increasing leptin release from adipocyte cells of a mammalian subject, comprising administering an FXR agonist to said subject in an amount effective to increase leptin release, compared to that which would occur in the absence of said FXR agonist administration.

2. A method of decreasing glucose uptake by adipocyte cells of a mammalian subject, comprising administering an FXR agonist to said subject in an amount effective to decrease glucose uptake compared to that which would occur in the absence of said FXR agonist administration.

3. A method of treating a mammalian subject in need of weight loss treatment, comprising administering to said subject a pharmaceutically acceptable FXR agonist in an amount effective to decrease said subject's weight, compared to the weight loss that would occur in the absence of FXR agonist treatment.

4. A method of reducing total body mass of a mammalian subject, comprising administering to a subject a pharmaceutically acceptable FXR agonist in an amount effective to reduce said subject's total body mass, compared to the subject's total body mass that would occur in the absence of FXR agonist treatment.

5. A method of increasing the metabolic rate of a mammalian subject, comprising administering to a subject a pharmaceutically acceptable FXR agonist in an amount effective to increase the metabolic rate of said subject, compared to the rate that would occur in the absence of FXR agonist treatment.

6. A method of increasing serum leptin in a mammalian subject, comprising administering to a subject a pharmaceutically acceptable FXR agonist in an amount effective to increase serum leptin in said subject, compared to that would occur in the absence of FXR agonist treatment.

7. A method according to any preceding claim, wherein said FXR agonist is selected from the group consisting of GW4064; 3α,7α-dihydroxy-6α-ethyl-5β-cholan-24-oic acid; 3α,7α-dihydroxy-6α-propyl-5β-cholan-24-oic acid; and 3α,7α-dihydroxy-6α-allyl-5β-cholan-24-oic acid, and pharmaceutically acceptable salts, solvates or amino acid conjugates thereof.

8. A method according to any preceding claim, where said subject is human.

9. A method according to any preceding claim, where said subject is a rodent.

10. A method according to any preceding claim, where said subject is a primate.

11. A method of inducing expression of FGF19 in a human hepatocyte cell, comprising administering an FXR agonist to said cell.

12. A method according to claim 11, wherein said FXR agonist is selected from the group consisting of GW4064; 3α,7α-dihydroxy-6α-ethyl-5β-cholan-24-oic acid; 3α,7α-dihydroxy-6α-propyl-5β-cholan-24-oic acid; and 3α,7α-dihydroxy-6α-allyl-5β-cholan-24-oic acid, and pharmaceutically acceptable salts, solvates or amino acid conjugates thereof.

13. A method according to claim 11 where said cell is *in vitro*.

SEQUENCE LISTING

<110> SmithKline Beecham Corporation
CuraGen Corporation

<120> Methods of Using Farnesoid X Receptor (FXR) Agonists

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IPM VCB

ENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To:
DAVID J. LEVY
GLAXOSMITHKLINE
FIVE MOORE DRIVE
PO BOX 13398
RESEARCH TRIANGLE PARK, NC 27709

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

	Date of Mailing (*day/month/year*) 16 AUG 2004
Applicant's or agent's file reference PU4692WO	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/US03/08634	International filing date (*day/month/year*) 19 March 2003 (19.03.2003)
Applicant SMITHKLINE BEECHAM CORPORATION	

1. ☒ The applicant is hereby notified that the international search report has been established and is transmitted herewith.

   Filing of amendments and statement under Article 19:
   The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

   When? The time limit for filing such amendments is normally two months from the date of transmittal of the international search report.

   Where? Directly to the International Bureau of WIPO, 34, chemin des Colombettes 1211 Geneva 20, Switzerland, Facsimile No.: (41-22) 740.14.35

   For more detailed instructions, see the notes on the accompanying sheet.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. ☐ **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

   ☐ the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

   ☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Reminders**

   Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90 *bis*.1 and 90 *bis*.3, respectively, before the completion of the technical preparations for international publication.

   Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase **until 30 months** from the priority date (in some Offices even later); otherwise the applicant must, **within 20 months** from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

   In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months.

   See the Annex to Form PCT/IB/301 and, for details about the applicable time limits, Office by Office, see the *PCT Applicant's Guide*, Volume II, National Chapters and the WIPO Internet site.

Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703)305-3230	Authorized officer Dorothea Lawrence For Marianne Seidel Telephone No. (703) 308-0196

Form PCT/ISA/220 (April 2002) *(See notes on accompanying sheet)*

Express Mail Label No.
EV330919064US

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference PU4692WO	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
International application No. PCT/US03/08634	International filing date (*day/month/year*) 19 March 2003 (19.03.2003)	(Earliest) Priority Date (*day/month/year*) 21 March 2002 (21.03.2002)
Applicant SMITHKLINE BEECHAM CORPORATION		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 3 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the Report**
   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
   ☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).
   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing:
   ☒ contained in the international application in written form.
   ☒ filed together with the international application in computer readable form.
   ☐ furnished subsequently to this Authority in written form.
   ☐ furnished subsequently to this Authority in computer readable form.
   ☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   ☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.
2. ☒ **Certain claims were found unsearchable** (See Box I).
3. ☐ **Unity of invention is lacking** (See Box II).
4. With regard to the **title**,
   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,
   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. ____
   ☐ as suggested by the applicant. ☐ None of the figures
   ☐ because the applicant failed to suggest a figure.
   ☐ because this figure better characterizes the invention.

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/US03/08634

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claim Nos.: 7-10
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US03/08634

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 31/42
US CL : 514/365

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/365

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WEST

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,321,036 A (SHER) 14 June 1994(14.06.1994), see entire document.	1-6, 11-13

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
29 July 2004 (29.07.2004)

Date of mailing of the international search report
16 AUG 2004

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
Facsimile No. (703)305-3230

Authorized officer
Marianne Seidel

Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under Article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the *PCT Applicant's Guide*, a publication of WIPO.

In these Notes, "Article," "Rule" and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions, respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended ?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Preliminary Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When ? Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments ?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How? Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments ?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped), whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under Article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international application is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments and any accompanying statement, under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the time of filing the amendments (and any statement) with the International Bureau, also file with the International Preliminary Examining Authority a copy of such amendments (and of any statement) and, where required, a translation of such amendments for the procedure before that Authority (see Rules 55.3(a) and 62.2, first sentence). For further information, see the Notes to the demand form (PCT/IPEA/401).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see the *PCT Applicant's Guide*, Volume II.